Comparison of the vasoconstrictor effects of the selective 5-HT_{1D}-receptor agonist L-775,606 with the mixed 5-HT_{1B/1D}-receptor agonist sumatriptan and 5-HT in human isolated coronary artery

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Aims Vasoconstriction in human coronary artery can be mediated via activation of both 5-HT₂ and 5-HT_{1B}-receptors. Coronary vasoconstriction is a rare, but potential adverse effect of the antimigraine drug sumatriptan. In order to investigate the receptor population involved we compared the vasoconstrictor effects of sumatriptan (a mixed 5-HT_{1B/1D}-receptor agonist) with those of L-775,606 (a selective 5-HT_{1D}-receptor agonist) and 5-HT (the endogenous ligand) in human isolated coronary arteries.

Methods Coronary arteries were obtained from human hearts removed prior to transplant surgery. Several endothelium denuded ring segments (4 mm in length) were obtained from each artery and mounted for isometric tension recording. Each segment was first exposed to 45 mM KCl and then to 5-HT (1 nM–100 µM). Concentration-effect curves to L-775,606 (1-(3-(5-(1,2,4-triazol-4-yl)-1H-indol-3-yl)propyl)-4-(2-(3-fluorophenyl)ethyl)piperazine) and sumatriptan were then performed in a consecutive and random manner. The response to repeated application of 5-HT was obtained in separate segments.

Results Twenty-five segments from seven different coronary arteries were studied. Concentration-effect curves were fitted to the data using nonlinear regression analysis. The maximum contraction for L-775,606 was significantly less than that for sumatriptan with $E_{\max}$ values (% relative to 45 mM KCl = 100%) of 30.1 ± 4.22 and 41.5 ± 2.7 , respectively. L-775,606 was significantly (30-fold) less potent than sumatriptan in causing contraction compared with sumatriptan (EC_{50} values were 6.0 µM and 0.2 µM, respectively). For comparison the $E_{\max}$ value for 5-HT was 77.2% and the EC_{50} value was 0.2 µM.

Conclusions The selective 5-HT_{1D}-receptor agonist L-775,606 has less propensity towards vasoconstriction in human isolated coronary artery (endothelium-denuded) than was mixed 5-HT_{1B/1D}-receptor agonist sumatriptan. The contractions produced were at concentrations where L-775,606 would be expected to occupy 5-HT_{1B}-receptors.

Keywords: 5-HT-receptor agonists, human coronary arteries, vasoconstriction

Introduction

Coronary side-effects, including isolated cases of cardiac ischaemia [1, 2] and myocardial infarction [3, 4], have been reported following sumatriptan administration. Although serious cardiac events following sumatriptan

administration are rare, such cases are of concern as it is possible that drugs of this class will be given unknowingly to migraine patients with concomitant silent ischaemic heart disease. It should be noted that the more common drug-related side-effect of 'chest symptoms' (including feelings of chest tightness, heaviness or pressure) experienced by some migraineurs is not cardiac in origin but may be related to changes in oesophageal motility or in the pulmonary vasculature [5, 6].

The antimigraine activity of 5-HT_{1B/1D}-receptor agonists is believed to be mediated through vasoconstriction of intracranial, extracerebral blood vessels and/or

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inhibition of the activation of trigeminal sensory nerves [7]. There is substantial evidence obtained using a variety of techniques (e.g. pharmacological characterization, RT-PCR, immunohistochemistry) to support the view that vasoconstrictor responses in human blood vessels (including coronary artery) are 5-HT_{1B}-receptor mediated [8–10]. In contrast, inhibition of transmitter release from the terminals of trigeminal nerves is 5-HT_{1D}-receptor mediated [11–13]. Thus a selective 5-HT_{1D}-receptor agonist may be effective in treating migraine headache but have reduced vasoconstrictor effects.

L-775,606 (1-(3-(5-(1,2,4-triazol-4-yl)-1H-indol-3-yl)propyl)-4-(2-(3-fluorophenyl)ethyl)piperazine) is a selective 5-HT_{1D}-receptor agonist. In cell lines expressing recombinant human 5-HT_{1D}- or 5-HT_{1B}-receptors, L-775,606 has been shown to have greater than 360-fold functional selectivity for 5-HT_{1D}-receptors compared with 5-HT_{1B}-receptors [14]. The aim of the present study was to compare the vasoconstrictor effects of L-775,606 on human isolated coronary artery with those of 5-HT and sumatriptan.

The collection of human material for these studies was approved by the University of Cambridge and Addenbrooke's Hospital Ethics Committee.

Methods

Patients

Coronary arteries (left descending, left circumflex or right coronary arteries) were obtained from seven patients (six males, one female) undergoing orthotopic cardiac transplant. The age of these patients ranged from 43 to 55 years. Reasons for cardiac transplant included dilated cardiomyopathy, cardiomyopathy secondary to viral myocarditis, ischaemic heart disease and left ventricular failure secondary to aortic valve disease.

Preparation of arterial segments

Arteries were dissected from the heart immediately following explantation. Five out of the seven arteries tested were kept overnight (4°C) in physiological salt solution control prior to use and these arteries were allowed to equilibrate at 37°C before commencing the experiment. The arteries were prepared by removal of excess connective tissue and then the endothelium was removed mechanically. Each artery was cut into segments (each segment was 4 mm in length) with 3, 4, 5 or 6 segments being prepared from each artery. Individual ring segments were mounted for isometric tension recording in tissue baths containing physiological salt solution (for composition see below) aerated with 95% O₂/5% CO₂ maintained at pH 7.4, 37°C. The segments were stretched in a stepwise manner and the maximum

length-tension curve was determined by measuring the response to 45 mM KCl and determining the point at which two different levels of stretch produced comparable responses to KCl. The segments were then allowed to equilibrate to their own basal tension level.

Experimental procedure

For all segments from the same artery, contractions to 45 mM KCl were first elicited and this response was used as a reference. To test for 5-HT responsiveness a cumulative concentration-effect curve to 5-HT was then obtained in all segments (Curve 1). The segments were then randomly allocated to one of three groups and the order of agonist application is shown in Table 1.

For each agonist, the response to each concentration was allowed to plateau (up to 10 min) before addition of the next concentration. If no response was observed after 3 min, the next concentration of the agonist was added to the organ bath. Further additions of agonists were suspended on attaining the maximum contractile response (i.e. where addition of a higher agonist concentration no longer produced a further increase in tension). Following each concentration-effect curve the segments were washed several times in control solution until baseline tone was re-established.

Drugs and solutions

5-HT was obtained from Sigma, UK. L-775,606 (maleate salt) and sumatriptan (succinate salt) were synthesized by Merck Sharp and Dohme Research Laboratories. The physiological salt solution had the following composition (mM): NaCl 90, KCl 5, MgSO₄·7H₂O 0.5, Na₂HPO₄ 1.0, NaCO₃ 45, glucose 10, CaCl₂ 2.5; Na pyruvate 5, fumarate 5.

Analysis of data

Responses to 5-HT, sumatriptan and L-775,606 were measured in g tension and expressed as a percentage of the response to 45 mM KCl. Inter-agonist comparisons were made by pooling data obtained in curves 2 and 3. All analyses were performed using BMDP statistical software (University of California Press, Los Angeles, Vax/VMS release 7.0, 1993).

Table 1 Order of agonist application.

	Group 1	Group 2	Group 3
Curve 1	5-HT	5-HT	5-HT
Curve 2	5-HT	Sumatriptan	L-775,606
Curve 3	5-HT	L-775,606	Sumatriptan

Comparison of overall effects of the agonists using analysis of variance (ANOVA)

ANOVAS were made on mean, standard error of mean, area of curve (AOC), linear slope values and all produced essentially similar results. Maximum and AOC, the variables judged to best describe the concentration-effect curves, are reported here. ANOVAS were performed to test for: (a) desensitization of responses to 5-HT obtained in curves 1, 2 and 3; (b) differences in agonist evoked responses across arteries obtained from different patients; (c) differences between the effects of 5-HT, sumatriptan and L-775,606 (curves 2 and 3).

Comparison of concentration-effect curve parameters ($E_{\max}$ and EC_{50} values) using nonlinear regression analysis

For each agonist, concentration-effect curves were fitted to the mean data, using weighted least squares nonlinear regression analysis and the equation $E = E_{\max} / (1 + (EC_{50}/\text{agonist concentration})^{nH})$, where $E_{\max}$ is the maximal contraction evoked by each agonist (relative to 45 mM KCl), EC_{50} is the half maximally effective concentration and nH is the Hill slope. The weighting was the reciprocal of the error estimate for each mean value. In all cases a good fit of the model was obtained and asymptotic 't' tests were used to contrast the $E_{\max}$ and EC_{50} values obtained for the agonists.

Results

A total of 25 arterial segments from seven donors were studied. Some of the arteries were stored overnight prior to use. It was anticipated that this would not effect the outcome of the experiment since the arteries were equilibrated at 37° C before starting the experiment and comparisons of the effects of the agonists were made using a 'within-segments' design. The response to 45 mM KCl was 5.36 ± 0.67 g. Desensitization to the 5-HT_{1B/1D}-receptor agonists was tested in a two-way ANOVA using the 5-HT data obtained in curves 1, 2 and 3. No statistically significant desensitization effect was found.

To test for interpatient variability in 5-HT responsiveness a two-way mixed design ANOVA was made in which the grouping factor was artery (patient) and the within subject factor was agonist concentration. A significant artery (patient) effect (Chi-square = 51.6, d.f. = 6, $P < 0.0001$) and interaction (with agonist concentration) effect (Chi square = 188.6, d.f. = 54, $P < 0.0001$) were detected, showing that interarterial differences go beyond random variation and confirms the value of within artery agonist comparisons.

Post ANOVA 't' tests (paired comparisons) showed that contractile responses to both sumatriptan and L-775,606

were significantly lower than those for 5-HT (maximums: $P = 0.023$ and $P < 0.0002$, respectively). For L-775,606 the area under the concentration-effect curve (AOC) was also significantly lower than that for 5-HT ($P < 0.0004$, for comparison sumatriptan *vs* AOC: $P = 0.0522$) and significantly lower than the AOC for sumatriptan ($P = 0.0412$).

Comparison of concentration-effect curves parameters ($E_{\max}$ and EC_{50} values)

Concentration-effect curves for 5-HT, sumatriptan and L-775,606 (using data obtained in curves 2 and 3) are shown in Figure 1 and the $E_{\max}$ and EC_{50} values are summarized in Table 2. Asymptotic 't' tests showed that the $E_{\max}$ values for both sumatriptan and L-775,606 were significantly lower than that for 5-HT (for 5-HT *vs* sumatriptan $t = 8.82$, d.f. = 12, $P < 0.00001$; the difference between the $E_{\max}$ values was 35.7% with 95% confidence interval (CI) 22.9, 48.5% and for 5-HT *vs* L-775,606 $t = 9.10$, d.f. = 13, $P < 0.00001$; the difference between the $E_{\max}$ values was 47.0% with 95% CI 30.9, 63.1%). The $E_{\max}$ value for L-775,606 was significantly less than that for sumatriptan ($t = 2.25$, d.f. = 13, $P < 0.021$; the difference between the $E_{\max}$ values was 11.3% with 95% CI -4.21, 26.8%). The EC_{50} value for sumatriptan was not significantly different from the value for 5-HT ($t = 0.24$, d.f. = 12, $P = 0.41$; the difference between the EC_{50} values was 0.03 with 95% CI -0.35, 0.41) whereas the EC_{50} value for L-775,606 was significantly different from the EC_{50} values for 5-HT ($t = 8.18$, d.f. = 13, $P < 0.00001$; the difference between the EC_{50} values was 1.52 with 95% CI 0.99, 2.06). The EC_{50} value for L-775,606 was

Figure 1 Concentration-effect curves to 5-HT (closed squares), sumatriptan (closed circles) and L-775,606 (open diamonds) obtained in isolated segments of endothelium-denuded human coronary artery. Points represent mean data expressed relative to the contraction evoked by 45 mM KCl. Vertical bars signify $\pm$ s.e. mean. Curves were fitted to the data using nonlinear regression analysis.

Table 2 Predicted $E_{\max}$ (% 45 mM KCl) and pEC_{50} values obtained from curve fitting to the overall mean data (obtained from Curves 2 and 3) using nonlinear regression analysis. The values shown represent mean with 95% confidence intervals (CI) (a total of 41 concentration-effect curves were performed on 25 segments obtained from arteries from seven different patients). Significantly different ($P < 0.03$) from 5-HT (*) and from 5-HT and sumatriptan (**).

	5-HT		Sumatriptan		L-775,606	
	pEC_{50}	$E_{\max}$	pEC_{50}	$E_{\max}$	pEC_{50}	$E_{\max}$
Mean	6.74 ⁺	77.2	6.71 ⁺	41.47*	5.22 ⁺ **	30.16**
95% CI	0.17	7.03	0.24	6.47	0.40	9.96

+ Corresponding to EC_{50} values of 0.2 μ M, 0.2 μ M and 6.0 μ M for 5-HT, sumatriptan and L-775,606, respectively.

significantly different from the value for sumatriptan ($t = 7.50$, d.f. = 13, $P < 0.00001$; the difference between the EC_{50} values was 1.49 with 95% CI 0.89, 2.09) showing that L-775,606 was 30-fold less potent in causing contraction than sumatriptan.

Discussion

In the present study, all arteries tested responded to 5-HT and, similarly to previous reports, there was significant variability in 5-HT responsiveness between arteries obtained from different patients [15]. These findings support the value of using a crossover design, with respect to order of agonist application, enabling the direct (within-segment) comparison of the vasoconstrictor effects of sumatriptan and L-775,606 on the same segment of human isolated coronary artery.

In human isolated coronary artery 5-HT-evoked vasoconstriction can be mediated via activation of both 5-HT_{1B}- and 5-HT₂-receptors [10, 16, 17]. The maximum contractile response evoked by both sumatriptan and L-775,606 was lower than that evoked by 5-HT which most likely reflects the relatively weak affinity of both of these drugs for human 5-HT₂-receptors [14]. The concentration-effect curve parameters for 5-HT and sumatriptan were in keeping with those previously reported [15]. Previously Nilsson *et al.* 1999 [18] have demonstrated the presence of 5-HT_{1B}-receptor immunoreactivity on the endothelial layer in normal coronary artery, however, the physiological role of these receptors is not clear. *In vitro* studies using isolated coronary arteries have shown that 5-HT receptor agonists produce similar effects in endothelium-denuded arterial segments obtained from diseased hearts [15] and in endothelium intact segments obtained from donor (normal) hearts [19]. However, it is possible that the *in vivo* situation may be different since the blood vessel would only be exposed to 5-HT_{1B/1D}-receptor agonists from the 'blood-side' and the endothelium may play a greater role in influencing coronary artery reactivity. Indeed, coronary angiographic studies have shown that in normal subjects 5-HT produces

a vasodilator response but in patients with coronary artery disease this response is lost and vasoconstriction is seen [20, 21]. 5-HT_{1B/1D}-receptor agonists are contraindicated in patients with known or suspected coronary artery disease.

The most important finding of the present study was the weak effect of L-775,606 on coronary artery reactivity. L-775,606 was markedly less potent than sumatriptan in causing contraction of human isolated coronary artery with the EC_{50} value for L-775,606 being 30-fold higher than the EC_{50} value for sumatriptan and in addition the maximum contraction was significantly lower than that of sumatriptan. L-775,606 is a selective 5-HT_{1D}-receptor agonist. In cell lines expressing human recombinant receptors L-775,606 is 360-fold times more potent in activating 5-HT_{1D}-receptors compared to 5-HT_{1B}-receptors (EC_{50} values of 1.2 nM and 440 nM, respectively [14]). For comparison, sumatriptan shows 16-fold selectivity for 5-HT_{1D}-over 5-HT_{1B}-receptors with EC_{50} values of 8.3 and 140 nM, respectively [14]. The weak potency of L-775,606 in causing coronary vasoconstriction is consistent with the activation of the 5-HT_{1B}-receptor subtype and furthermore immunohistochemical studies in human coronary artery have shown the selective expression of 5-HT_{1B}-receptor protein, but not 5-HT_{1D}-receptor protein in this artery [10].

In clinical trials it has been shown that clinically relevant oral doses of sumatriptan (100 mg) and rizatriptan (10 mg) produce plasma levels of 47.4 ng ml⁻¹ and 19.8 ng ml⁻¹ (equivalent to 160 nM and 56 nM, respectively [22]). These plasma levels would be expected to activate peripheral 5-HT_{1D}-receptors located on trigeminal nerve terminals surrounding blood vessels within the dura mater [11]. The EC_{50} at recombinant human 5-HT_{1D}-receptors is approximately 8 nM for both drugs [23]). Since L-775,606 has eightfold higher potency at the 5-HT_{1D}-receptor the predicted therapeutic plasma levels of free L-775,606 would be between 9 and 20 nM (assuming the antimigraine effect of sumatriptan and rizatriptan is mediated via 5-HT_{1D}-receptor activation). Thus for L-775,606 there is greater than a 300-fold

window between the EC_{50} value ($6\ \mu\text{M}$) in coronary artery and the predicted therapeutic plasma levels of L-775,606. This is a substantial improvement over existing triptans. Recently, Maassen VanDenBrink *et al.* [19] have made similar comparisons for a series of triptans between EC_{50} values in isolated coronary artery and therapeutically relevant plasma levels (C_{max}), showing some apparent differences between triptans. However, small differences in EC_{50} values (obtained as part of a logarithmic dose-response relationship) can produce large differences in the EC_{50}/C_{max} ratio. For example, in the study by Maassen VanDenBrink *et al.* [19] the EC_{50} for zolmitriptan was 478 nM (equivalent to $pEC_{50}=6.3$), giving a EC_{50}/C_{max} ratio of 0.02 whereas Nilsson *et al.* [18] reported an EC_{50} value of 251 nM ($pEC_{50}=6.5$) for zolmitriptan giving a EC_{50}/C_{max} ratio of 0.1 (compared with a ratio of 0.18 for sumatriptan). Therefore, apparent differences between triptans currently used as antimigraine drugs should be viewed with caution, since the differences may be small and all drugs will carry similar cardiac risk liability. Selective 5-HT_{1D}-receptor agonists, such as L-775,606 may offer a real advantage since vasoconstrictor effects in human isolated coronary artery are small in magnitude and the window between coronary vasoconstriction and predicted therapeutic plasma levels is greater than 300-fold (EC_{50}/C_{max} ratio = <0.003). The efficacy of selective 5-HT_{1D}-receptor agonists as antimigraine agents needs to be established.

In conclusion, L-775,606 (a selective 5-HT_{1D}-receptor agonist) was markedly less potent than sumatriptan (a mixed 5-HT_{1B/1D}-receptor agonist) in causing contraction of human isolated coronary artery. This observation is consistent with the suggestion that vasoconstriction in human coronary artery is mediated via the 5-HT_{1B}-receptor subtype and with the view that selective 5-HT_{1D}-receptor agonists are likely to have reduced vasoconstrictor properties in man.

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